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(54) Title: NOVEL DIPEPTIDE DERIVATIVES

(57) Abstract

Pharmaceutically active compounds covalently bound to a dipeptide residue and the use of the same for increasing the uptake of the pharmaceutically active compounds in medicine.

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NOVEL DIPEPTIDE DERIVATIVES

Field of the invention

The present invention relates to the use in therapy of dipeptide moieties covalently coupled to drugs which are not α-amino acids or peptides and which contain a carboxylic group, thereby forming prodrugs for the parent drugs. Possibly the dipeptide moieties function as uptake enhancers in the gastrointestinal tract of mammals including man. The use of the derivatives is especially valuable for the enhancement of the uptake of drugs which are charged at the pH in the gastrointestinal tract. In particular the present invention relates to the use of derivatives of phosphonoformic acid (PFA) as prodrugs in pharmaceutical compositions.

The present invention also relates to novel dipeptide derivatives of pharmaceutically active compounds, which are not α-amino acids or peptides and which contain a carboxyl group, as prodrugs. In particular the present invention relates to derivatives of PFA and the use thereof as prodrugs in pharmaceutical compositions. The PFA derivatives can be formulated for oral administration resulting in high bioavailability as measured by the level of PFA in the blood upon ingestion.

The present invention also relates to processes for the preparation of the PFA derivatives.

The present invention also relates to a method of improving the transport of pharmaceutically active compounds, which are not α-amino acids or peptides and which contain a carboxyl group, via the intestinal mucosa into the blood of mammals. The improved transport is accomplished by chemical modification of the parent drug with a bioreversible prodrug moiety which is disclosed in the present invention and which mediates the active uptake of the parent drug. PFA is a parent

drug which is particularly suitable for improved transport according to this method.

The present invention also relates to a method for the therapeutic and prophylactic control and treatment of viral infections in humans. These include infections caused by all human herpesviruses, including cytomegalovirus (CMV), herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), Epstein-Barr virus (EBV), varicella zoster virus (VZV), human herpesvirus 6 (HHV-6) as well as by human immunodeficiency virus (HIV).

Definitions

The following abbreviations and definitions will be used hereinafter.

The abbreviation "i.v." stands for "intravenous".

The abbreviation "p.o." stands for "peroral".

The abbreviation "GI" stands for "gastrointestinal".

The abbreviation "PFA" stands for "phosphonoformic acid".

The abbreviation "DEPFA" stands for "di-(O-ethyl)phosphonoformic acid".

The abbreviation "DEPF-GlyPro" stands for the derivative of DEPFA with the dipeptide GlyPro.

The term "foscarnet" is defined herein as foscarnet sodium, the hexahydrate of the trisodium salt of phosphonoformic acid.

The term "bioavailability" is defined herein as the fraction of an oral dose that reaches the systemic circulation.

The term "C_{max}" is defined herein as the peak or highest drug concentration in the systemic circulation.

The term "AUC" is defined herein as the total integrated area under the concentration/time curve. It is an estimate of the amount of drug absorbed.

The term "CMV end-organ disease" is defined herein as the disease associated with any organ infected by cytomegalovirus.

"F_{abs}" is the fraction of the drug absorbed which enters into the blood, i.e. $AUC_{oral}/AUC_{i.v.}$.

Background of the Invention

Intravenous formulations of PFA are well known and are disclosed in U.S. Patent Nos. 4,215,113; 4,339,445; 4,665,062 and 4,771,041.

PFA inhibits replication of all known herpesviruses in vitro including cytomegalovirus (CMV), herpes simplex virus types 1 and 2 (HSV-1, HSV-2), human herpesvirus 6 (HHV-6), Epstein-Barr virus (EBV) and varicella-zoster virus (VZV) as well as certain retroviruses including the human immunodeficiency virus (HIV) types 1 and 2 (HIV-1 and HIV-2).

Treatment of CMV infections in AIDS patients and patients infected with herpesvirus with foscarnet is at present by intravenous injections. This mode of treatment is burdensome where foscarnet must be administered daily. The development of an oral formulation is therefore very desirable since it would offer a much more convenient method of treatment and thus result in easier, more reliable compliance. While oral formulations of PFA have been tested there is no known proven effective composition available on the market to date.

The oral administration of an aqueous solution of intravenous foscarnet in animals leads to reduced and inconsistent absorption from the GI tract and therefore, low bioavailabilities and low peak blood levels, e.g. in the dog (Ritschel et al., 1985, Meth. Exptl. Clin. Pharmacol. 2:41-48).

One explanation of the low bioavailability might be that PFA is absorbed poorly in the intestine because of its being charged at the pH of the GI tract.

Another explanation of the low bioavailability might be that PFA is rapidly degraded in the stomach by the inherent low pH as the drug is acid labile. This acid lability may, at least in part, account for the lower amount of PFA available for absorption, and therefore, the low bioavailabilities previously attained. The studies of Ritschel et al. (1985) showed that the absorption of PFA in an animal having a stomach pH close to neutral (the rabbit), is much better than in one with an acidic stomach pH (the dog), thus resulting in a higher bioavailability. Bundgaard et al. (Int. J. Pharm. 63, 1990, 213-218) studied the decarboxylation of foscarnet in acidic solution and concluded that intragastric degradation might be of significance for the absorption of foscarnet upon peroral administration.

All attempts in the past to administer oral foscarnet to human subjects using the currently approved intravenous formulation have been suboptimal and thus, unsuccessful. (Sjövall et al., 1988, Clin. Pharm. Ther., 44:65-73 and Barditch-Croyo et al. 1991 7th Intl. Conf. on AIDS, Florence, Italy).

Alkyl derivatives of phosphonoformic acid are known from EP 0 003 007 as are the antiviral effects in vitro and in vivo in animals of such compounds and of pharmaceutical compositions thereof. So far, however, no drug based on any of these substances has become available neither in oral nor in any other formulation.

Amide derivatives of phosphonoformic acid are known from EP 0 003 008 as are the antiviral effects in vitro and in vivo in animals of such compounds and of

pharmaceutical compositions thereof. So far, however, no drug based on any of these substances has become available neither in oral nor in any other formulation.

Prodrugs of phosphorus derivatives which are designed for penetrating the blood brain barrier have been described in Eur. pat. appl. No. 91912398.4 (Glazier, A.).

An objective of the present invention is to provide a novel derivative of PFA in a suitable oral formulation which can be administered to humans and can deliver high concentrations of PFA in the blood of patients.

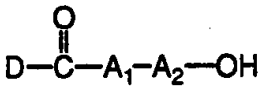
Another objective of the present invention relates to the use of a chemical modification of PFA to be delivered via the intestinal mucosa into the blood as a means of improving the uptake of PFA.

It is a further objective of the present invention to provide formulations of derivatives of PFA which exhibit acceptable absorption rates, and high and effective bioavailability.

Another objective of the present invention relates to the use of an effective amount of flavoring agent to provide the level of flavor desired to mask the taste of the oral formulations of prodrugs of PFA.

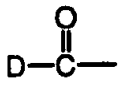
Summary of the Invention

In a general aspect the invention comprises a compound of the general formula I



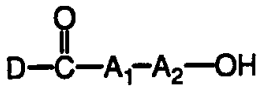
I

wherein



is a radical of a pharmaceutically active compound, D-COOH which is not an α-amino acid or peptide and which is able to form an amide bond with the N-terminal group of a dipeptide H-A_1-A_2-OH as defined below.

The invention also comprises pharmaceutical compositions containing a compound of the general formula I

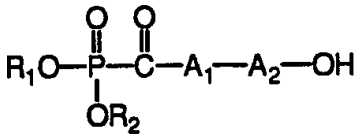


I

as an active ingredient and the use of such compositions of the compounds in the therapy of the diseases which are appropriate for treatment by the pharmaceutically active compound.

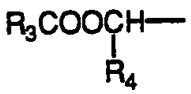
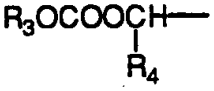
The present invention results from the following unexpected finding: A dipeptide derivative of di-(O-ethyl)phosphonoformic acid formulated and administered in aqueous saline solution to rats is actively taken up and bioconverted to release high levels of PFA into the blood.

The dipeptide derivatives of PFA according to the present invention have the general formula II

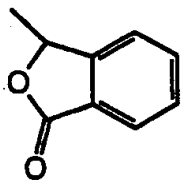


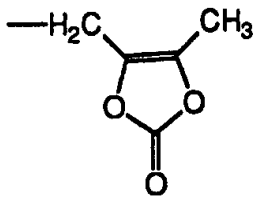
II

wherein R_1 and R_2 each independently are hydrogen; a straight or branched C_{1-6}-alkyl, C_{2-6}-alkenyl, C_{2-6}alkynyl, C_{3-8}-cycloalkyl, C_{3-8}-cycloalkyl-C_{1-6}-alkyl or C_{1-6}-alkoxy-C_{1-6}-alkyl group which is optionally substituted with hydroxy, amino, halogen or oxo; a benzyl group;

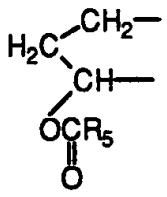
a group  or a group 

wherein R_3 is a straight or branched C_{1-6}-alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-8}-cycloalkyl, C_{3-8}-cycloalkyl-C_{1-6}-alkyl or C_{1-6}-alkoxy-C_{1-6}-alkyl group which is optionally substituted with hydroxy, amino, halogen or oxo, and R_4 is hydrogen or a C_{1-4}-alkyl group;

a group  ;

a group  ;

or wherein R_1 and R_2 together form a group



wherein R_5 is a straight or branched C_{1-6}-alkyl or C_{1-6}-alkoxy group;

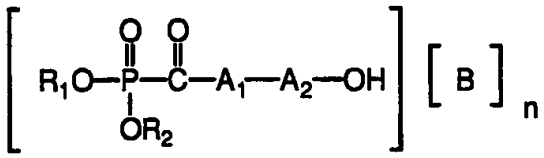
A_1 is an amino acid residue which is selected from glycyl, alanyl, valyl, norvalyl, leucyl, isoleucyl, norleucyl, phenylalanyl, tyrosyl, seryl, homoseryl, threonyl, cysteinyl, methionyl, tryptophyl, α-aspartyl, α-glutamyl, arginyl, lysyl, histidyl, ornithyl, prolyl or 4-hydroxyprolyl, either in the L- or in the D-configuration;

A_2 is an amino acid residue which is selected from prolyl, 4-hydroxyprolyl, phenylalanyl or tyrosyl, either in the L- or in the D-configuration; or physiologically acceptable salts thereof.

Examples of metal salts which can be prepared are salts containing Li, Na, K, Ca, Mg, Zn, Mn and Ba. A less soluble metal salt can be precipitated from a solution of a more soluble salt by addition of a suitable metal compound. Thus for examples, Ca, Ba, Zn, Mg, and Mn salts of the active substances can be prepared

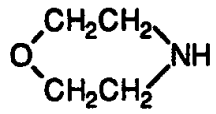
from sodium salts thereof. The metal ion of a metal salt of the active substances can be exchanged by hydrogen ions, other metal ions, ammonium ion and ammonium ions substituted by one or more organic radicals by using a cation exchanger.

Examples of other useful salts which can be prepared in this way are the salts of the formula



in which formula R_1, R_2, A_1 and A_2 have the same meaning as above, n is 1 or 2, and B is a salt-forming component such as

NH_3, CH_3NH_2, $C_2H_5NH_2$, $C_3H_7NH_2$, $C_4H_9NH_2$, $C_5H_{11}NH_2$, $C_6H_{13}NH_2$, $(CH_3)_2NH$, $(C_2H_5)_2NH$, $(C_3H_7)_2NH$, $(C_4H_9)_2NH$, $(C_5H_{11})_2NH$, $(C_6H_{13})_2NH$, $(CH_3)_3N$, $(C_2H_5)_3N$, $(C_3H_7)_3N$, $(C_4H_9)_3N$, $(C_5H_{11})_3N$, $(C_6H_{13})_3N$, $C_6H_5CH_2NH_2$, $HOCH_2CH_2NH_2$, $(HOCH_2CH_2)_2NH$, $(HOCH_2CH_2)_3N$, $C_2H_5NH(CH_2CH_2OH)$, $C_2H_5N(CH_2CH_2OH)_2$, $(HOH_2C)_3CNH_2$ and



Further examples of other useful salts which can be prepared by the ion exchange technique are quaternary ammonium salts of the active substances, i.e. salts in which the hydrogens in the active substances (structural formulas I and II) have been substituted with quaternary ammonium ions such as $(CH_3)_4N^+$, $(C_3H_7)_4N^+$, $(C_4H_9)_4N^+$, $(C_5H_{11})_4N^+$, $(C_6H_{13})_4N^+$ and $C_2H_5N(CH_2CH_2OH)_3$. Lipophilic

salts of this type can also be prepared by mixing salts of the active substances with a quaternary ammonium salt in water and extracting out the resulting quaternary ammonium salt of the active substance with an organic solvent such as dichloromethane, chloroform, ethyl acetate or methyl isobutyl ketone.

The derivatives of PFA of the present invention are effective for the treatment of HIV infections and human herpesvirus infections by inhibiting the replication of the human immunodeficiency virus (HIV-1 and HIV-2), cytomegalovirus (CMV), herpes simplex virus types 1 and 2 (HSV-1 and HSV-2), Epstein-Barr virus (EBV), varicella zoster virus (VZV) and human herpesvirus 6 (HHV-6).

Detailed description of the invention

The compounds of the invention are dipeptide derivatives of drugs which are not α-amino acids or peptides and which contain a carboxylic group which can form an amide bond with the N-terminal end of dipeptides. Most preferred are dipeptide derivatives with a L-proline, L-4-hydroxyproline L-phenylalanine or L-tyrosine residue at the C-terminal end. Most preferred N-terminal amino acid residue is glycyl or L-alanyl.

The present invention provides compounds which are the dipeptide derivatives of pharmaceutically active compounds which are not α-amino acids or peptides and which contain a carboxyl group which can form an amide bond to an amino acid residue. Most preferred are dipeptide derivatives with a L-proline, L-4-hydroxyproline, L-phenylalanine or L-tyrosine residue at the C-terminal end. Most preferred N-terminal amino acid residue is glycyl or L-alanyl. In particular the present invention relates to novel derivatives of PFA in which a dipeptide is attached to the carboxyl group of PFA by means of an amide bond.

The present invention also provides a method of enhancing the uptake of PFA and derivatives thereof into the blood by coupling a dipeptide via an amide bond

thereto.

The present invention also provides:

A. A method for treatment of diseases caused by viruses in animals, including man, comprising administering to an animal so infected a therapeutically effective amount of a compound of the formula II or a physiologically acceptable salt thereof.

B. A method for the treatment of diseases caused by viruses in animals, including man, by inhibiting the activity of viral polymerase, characterized by administering to an animal so infected a compound of the formula II or a physiologically acceptable salt thereof in an amount effective for inhibiting the activity of said viral polymerase.

C. A method for inhibiting the activity of reverse transcriptases of viruses in animals, including man, by administration to an animal a compound of the formula II or a physiologically acceptable salt thereof in an amount sufficient for inhibiting the activity of said reverse transcriptase. Particular reverse transcriptases are the reverse transcriptases of retroviruses, such as visna, sarcoma and leucemia viruses, and human immunodeficiency virus (HIV).

D. A method for inhibiting the multiplication of virus, in particular herpesviruses, influenza virus and hepatitis B virus, and retroviruses in animals, including man, by administering to an animal in need of such treatment a compound of the formula II or a physiologically acceptable salt thereof in an amount sufficient for inhibiting said multiplication.

E. A method for inhibiting the growth of virus-transformed cells in animals, including man, characterized by administering to an animal in need of such treatment a compound of the formula II or a physiologically acceptable salt thereof

in an amount sufficient for inhibiting said growth.

The invention also relates to the use of a compound of the formula II or a physiologically acceptable salt thereof, in each of the above given methods A, B, C, D and E. For example, the invention relates to the use of a compound of the formula I or a physiologically acceptable salt thereof, for

a) inhibiting the replication of viruses in animals including man, in particular herpesviruses including CMV, influenza virus, hepatitis B viruses and human immunodeficiency virus.

b) for inhibiting the growth of virus-transformed cells in animals including man.

Furthermore, the invention provides pharmaceutical preparations comprising as active ingredient a compound of the formula II or a physiologically acceptable salt thereof, optionally in association with a pharmaceutically acceptable carrier. The invention also encompasses a process for the preparation of a medicine having antiviral acitivity, characterized in that a compound of the formula II or a physiologically acceptable salt thereof is brought into an administration form suitable for therapeutical purposes, and the shaped medicine obtained by such process.

The derivatives of PFA or salts thereof according to the invention may be used for the therapeutic and prophylactic control and treatment of herpesvirus diseases and HIV diseases. Oral formulations of the prodrugs of PFA or salts thereof can be used alone or with other antiviral agents such as acyclovir, ganciclovir, ddC, ddI, AZT or immunological agents such as interferon and growth factors such as granulocyte-macrophage and granulocyte-colony stimulating factors (GM-CSF and G-CSF).

Other drugs, which can be derivatized and used according to the present invention

are analgesics, antirheumatics and antiphlogistics, such as acemetacin, acetylsalicylic acid, alclofenac, diclofenac, diflunisal, fenoprofen, flurbiprofen, ibuprofen, indomethacine, ketoprofen, ketozolac, naproxen, niflumic acid, oxaprozin, pirprofen, salicylic acid, sulindac, tiaprofenic acid, tolfenamic acid and tolmetin; antibacterials and antivirals such as cinoxacin, ciprofloxacin, nalidixic acid and phosphonoacetic acid; diuretics such as etacrynic acid and canrenoic acid; hemostatics such as tranexamic acid; oncolytics such as chlorambucil; prostaglandins, such as alprostadil, carboprost, dinoprost, dinoprostone and epoprostenol; and thyreomimetics such as levothyroxine and liothyronine.

In the present context, the terms "C_{1-4}-alkyl" and "C_{1-6}-alkyl" as a separate group or as part of a group designates alkyl groups with 1-4 or 1-6 carbon atoms which may be straight or branched such as methyl, ethyl, propyl, isopropyl, butyl, isobutyl, tert-butyl, pentyl, hexyl etc. The term "C_{2-6}-alkenyl" designates mono-unsaturated alkyl groups with 2-6 carbon atoms which may be straight or branched, preferably straight, in which the double bond may be present anywhere in the chain, for example vinyl, 1-propenyl, 2-propenyl, 1-butenyl, 2-butenyl, 3-butenyl etc. The term C_{2-6}-alkynyl" designates an alkyl group with 2-6 carbon atoms and incorporating a triple bond, e.g. ethynyl, 1-propynyl, 2-propynyl, 2-butynyl, etc. The term "C_{3-8}-cycloalkyl" as a group or as part of a group designates a cyclic alkyl group with 3-8 carbon atoms such as cyclopropyl, cyclobutyl, cyclopentyl, cyclohexyl, cycloheptyl or cyclooctyl. The term "C_{1-6}-alkoxy" designates groups comprising an oxa function substituted with an alkyl group as defined above. The term "C_{3-8}-cycloalkoxy" designates groups comprising an oxa function substituted with a cycloalkyl group as defined above. The term "halogen" designates Cl, Br, I and F.

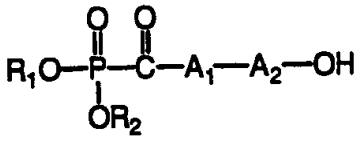
Preferred groups of the radicals R_1 and R_2 in the formula I below are ethyl, propyl, isopropyl, acetoxymethyl, acetoxyethyl, pivaloyloxymethyl, 1-(ethoxycarbonyloxy)ethyl, phthalidyl and (5-methyl-2-oxo-1,3-dioxolen-4-yl)methyl. Preferred amino acid residues A_1 are glycyl and L-alanyl and preferred amino acid

residues A_2 are L-phenylalanyl, L-tyrosyl, L-prolyl and L-4-hydroxyprolyl.

Preparation

Reference to "meaning given above" for R_1, R_2, A_1 and A_2 as used below refers to the definitions given in formula II.

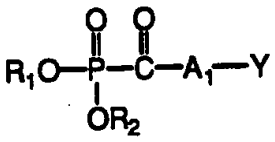
Compounds of the general formula II



II

wherein R_1, R_2, A_1 and A_2 are as defined above are prepared by known methods for the synthesis of carbamoylphosphonates, for example as described by T. Reetz et al in J. Am. Chem. Soc. 77 (1955) 3813 and in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XII/1, Organische Phosphorverbindungen, p. 453-458. Examples of such methods are the following.

A. Reacting a compound of the formula III



III

with an amino acid derivative H-A_2-OR_6 wherein R_1, R_2, A_1 and A_2 have the meaning given above, R_6 is a suitable carboxyl protecting group as described, for example, in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XV/1, Blockierung und Schutz der α-Carboxy-Funktion, p. 315-450, such as

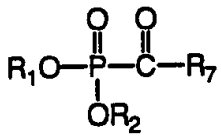
methyl, ethyl, or benzyl, and Y is a hydroxyl group or a carboxyl-activating group as is used in the art of peptide synthesis, as described for example in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XV/2, Die Herstellung der Peptidbindung, p. 1-453, followed by removal of the protecting group to give a compound of the formula II. Examples of carboxyl-activating groups Y are azide, substituted aryl, such as pentachlorophenoxy or 4-nitrophenoxy, substituted hydroxylamines such as succinimidoxy and benzotriazol-1-oxy and azolyls such as imidazolyl. The carboxyl-activating group may also be introduced in situ by carrying out the peptide-forming reaction in the presence of a carbodiimide, such as dicyclohexyl carbodiimide. Another method of activating the carboxyl group is by formation of a mixed anhydride, for example by reaction with ethyl chloroformate.

The protecting group R_6 may be removed by hydrolysis with a base such as, for example, 0.5M - 2M sodium hydroxide, lithium hydroxide or potassium hydroxide in water, methanol, ethanol or aqueous tetrahydrofuran. The protecting group R_6 may also be removed by enzymatic hydrolysis, for example with porcine liver esterase. Examples of such methods are described, for example, by H. G. Davies et al in Best Synthetic Methods; Biotransformations in Preparative Organic Chemistry; Academic Press; London, 1989; Chapter 2.

When the protecting group R_6 is benzyl, it may be removed by catalytic hydrogenation in the presence of a catalyst, such as palladium on charcoal.

The phosphonates of formula III where Y = OH are prepared according to the methods for the synthesis of carbamoylphosphonates, for example as described by T. Reetz et al. in J. Am.Chem. Soc. 77 (1955) 3813 and in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XII/1, Organische Phosphorverbindungen, p. 453-458.

B. Reacting a compound of the formula IV

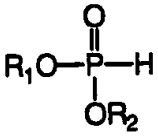


IV

wherein R_1 and R_2 have the meaning given above and R_7 is an aliphatic cycloaliphatic, araliphatic, aromatic or heterocyclic leaving group, such as C_{1-6}-alkoxy, C_{3-8}-cycloalkoxy, benzyloxy, phenoxy, 4-nitrophenoxy, imidazolyl or succinimidoxy with a dipeptide derivative H-A_1-A_2-OR_6, wherein A_1, A_2 and R_6 have the meaning given above followed by removal of the protecting group R_6, as described above, to give a compound of the formula II.

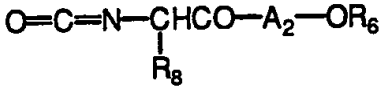
Preferentially the carbamoylphosphonate-forming reaction is performed in a solvent, such as for example ethanol or dimethylformamide, at a temperature from 0°C to 100°C for 1 hour to 5 days.

C. Reaction of a compound of the formula V



V

wherein R_1 and R_2 have the meaning given above, with a compound of the formula VI



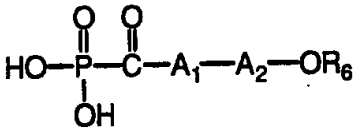
VI

wherein A_2 and R_6 have the meaning given above and R_8 is the side chain specific for an amino acid residue A_1 as defined in claim 1, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II.

Preferentially the carbamoylphosphonate-forming reaction is performed at 50°C to 150°C for 1 to 50 hours.

The starting materials used in the above methods of preparation B and C are known compounds, or may be prepared by known methods commonly used for the synthesis of hydroxycarbonylphosphonic acid triesters, phosphite esters, isocyanates and esters of amino acids and peptides. Examples of methods for the synthesis of phosphite esters may be found in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XII/2, Organische Phosphorverbindungen, p. 5-78. Examples of methods for the synthesis of hydroxycarbonylphosphonic acid triesters are found in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XII/1, Organische Phosphorverbindungen, p. 433-463. Examples of methods for the synthesis of isocyanates are found in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band E4, Isocyanate, p. 738-834. Examples of methods for the synthesis of esters of amino acids and peptides are found in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XV/1, p. 315-405.

D. Esterification of a compound of the formula VII



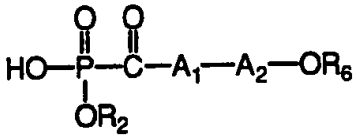
VII

with an alcohol R_1OH, wherein A_1, A_2, R_1 and R_6 have the meaning given above, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II where R_1=R_2.

The esterification reaction is performed through the intermediary of activating agents known per se for the phosphorylation of alcohols. Examples of such methods are described for example by L. A. Slotin in Synthesis 1977, 737 and by H. Seliger and H. Kössel in Progress in the Chemistry of Organic Natural Products 32 (1975) 297.

Synthesis of the phosphonic acids of the formula VII are described below in methods K and L.

E. Esterification of a compound of the formula VIII

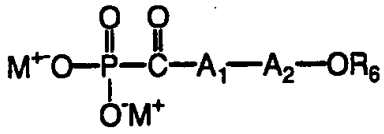


VIII

with an alcohol R_1OH, wherein A_1, A_2, R_1, R_2 and R_6 have the meaning given above, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II.

The esterification reaction is performed through the intermediary of activating agents known per se for the phosphorylation of alcohols. Examples of such methods are described for example by L.A. Slotin in Synthesis 1977, 737 and by H. Seliger and H. Kössel in Progress in the Chemistry of Organic Natural Products 32 (1975) 297. Synthesis of the phosphonic acid monoesters of formula VI are described below in methods M-O.

F. Reaction of a compound of the formula IX

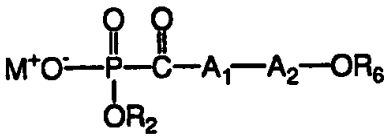


IX

with a compound R_1-X, wherein A_1, A_2, R_1 and R_6 have the meaning given above, M^+ is a cation such as Ag^+, Li^+, Na^+, K^+, Cs^+, Et_3NH^+, $(i\text{-}Pr)_2NEtH^+$ and X is a halogen such as Cl, Br or I, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II where R_1=R_2.

Preferentially the esterification reaction is carried out in a solvent, such as for example ethanol or dimethylformamide, at a temperature from 25°C to 100°C for 1 to 50 hours.

G. Reaction of a compound of the formula X



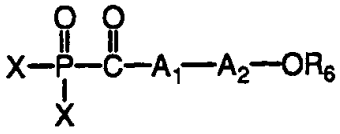
X

with a compound R_1-X, wherein A_1, A_2, R_1, R_2, R_6, M^+ and X have the meaning given above, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II.

Preferentially the esterification reaction is carried out in a solvent, such as for example ethanol or dimethylformamide, at a temperature of 25°C to 100°C for 1 to 50 hours.

H. Reacting a compound of the formula XI

XI



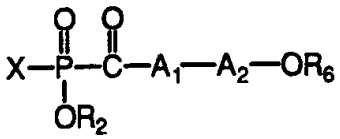
with an alcohol R_1OH,

wherein A_1, A_2, R_1, R_6 and X have the meaning given above, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II where $R_1=R_2$.

The esterification reaction is performed by methods known per se for the phosphorylation of alcohols by phosphoric and phosphonic acid halides. Examples of such methods are described for example by L.A. Slotin in Synthesis 1977, 737 and by H. Seliger and H. Kössel in Progress in the Chemistry of Organic Natural Products 32 (1975) 297.

The dihalides of the formula XI are prepared from the corresponding phosphonic acids by methods known per se for the synthesis of dihalides of phosphonic acids and phosphoric acids. References for those methods are found for example in the two publications above and in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XII/1, p. 386-406 and Band XII/2, p. 211-225 and p 274-292. The phosphonic acids are prepared by methods described in methods K and L.

J. Reacting a compound of the formula XII



XII

with an alcohol R_1OH,

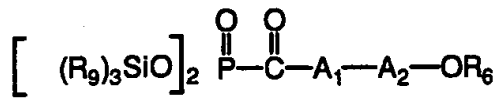
wherein A_1, A_2, R_1, R_2, R_6 and X have the meaning given above, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II.

The esterification reaction is performed by methods known per se for the phosphorylation of alcohols. Examples of such methods are described for example by L.A. Slotin in Synthesis 1977, 737 and by H. Seliger and H. Kössel in Progress in the Chemistry of Organic Natural Products 32 (1975) 297.

The monoester halides of the formula XII are prepared from the corresponding phosphonic acid monoesters by methods known per se for the synthesis of monohalides of phosphonic and phosphoric acids. References for those methods are found for example in the two publications above and in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XII/1, p. 386-406 and Band XII/2, p. 211-225 and p. 274-292.

The corresponding phosphonic acid monoesters are prepared by methods described below in M-O.

K. Aqueous hydrolysis of a compound of the formula XIII containing two silylated phosphonate groups

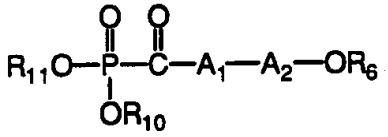


XIII

wherein A_1, A_2 and R_6 have the meaning given above, and R_9 is an inert organic residue, for example methyl, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II where R_1=R_2=H.

Optionally, the phosphonic acid groups formed on hydrolysing the bis-silyl esters can be neutralized. Preferably they can be neutralized with a weak cation exchanger (M^+) or with a base such as $MHCO_3$, M_2CO_3 or MOH. M^+ is NH_4^+ or a metal cation such as Li^+, Na^+ or K^+.

The phosphonate bis-silyl esters may be obtained by reacting a compound of the formula XIV



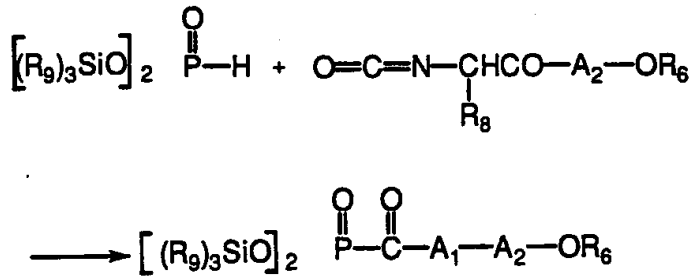
XIV

with a compound X-Si(R_9)$_3$, wherein R_6, R_9, A_1, A_2 and X have the meaning given above and R_{10} and R_{11} have the meaning given R_1 and R_2. R_{10} and R_{11} may be the same or different.

Preferentially, the reaction is performed at -20°C to reflux temperatures for 1 hour to several days.

The phosphonic acid diesters of formula XIV are prepared by methods analagous to those described in A-J.

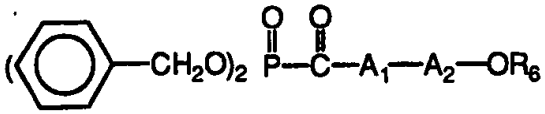
Alternatively, the phosphonate bis-silyl esters may be prepared by reacting a bis-silyl phosphite with an isocyanate according to the formula



where R_6, R_8, R_9, A_1 and A_2 have the meaning given above. Preferentially the reaction is performed at 25°C to 150°C for 1 to 50 hours.

The bis-silyl phosphites are prepared by known methods, as described for example by Sekine et al in J. Org. Chem. 46 (1981) 2097, for the preparation of bis(trimethylsilyl) phosphite.

L. Hydrogenation of a compound of the formula XV

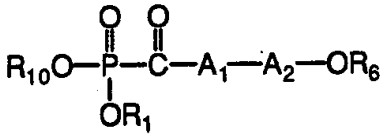


XV

wherein A_1, A_2 and R_6 have the meaning given above, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II where R_1=R_2=H.

Preferably the hydrogenation reaction may be performed with a catalyst such as palladium on charcoal. Optionally the phosphonic acid groups may be neutralized. Preferably they may be neutralized with a weak cation exchanger (M^+) or with a base such as $MHCO_3$, M_2CO_3 or MOH. M^+ is for example NH_4^+ or a metal cation such as Li^+, Na^+ or K^+.

M. Reacting a compound of the formula XVI



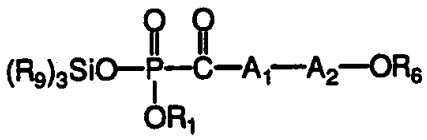
XVI

with iodide or bromide anion, wherein A_1, A_2, R_1, R_6 and R_{10} have the meaning given above followed by removal of the protecting group R_6, as described above, to give a compound of the formula II where R_2=H.

Preferably the reaction is carried out with sodium iodide in a solvent such as for example tetrahydrofuran or acetone. Preferably the reaction is carried out at a temperature from 20°C to 100°C from 2 hours to 7 days.

The phosphonic acid diesters of formula XVI may be prepared by methods analogous to those described in A-J.

N. Aqueous hydrolysis of a compound of the formula XVII containing one silylated phosphonate group



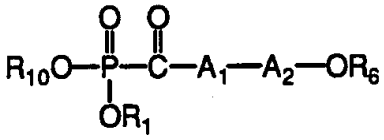
XVII

wherein A_1, A_2, R_1, R_6 and R_9 have the meaning given above, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II where R_2=H.

Optionally the phosphonic acid group formed on hydrolysing the silyl ester may be

neutralized. Preferably it may be neutralized with a weak cation exchanger (M^+) or with a base such as $MHCO_3$, M_2CO_3 or MOH. M^+ is for example NH_4^+ or a metal cation such as Li^+, Na^+ or K^+.

The silyl esterified phosphonate group may be obtained by reacting a compound of the formula XVI

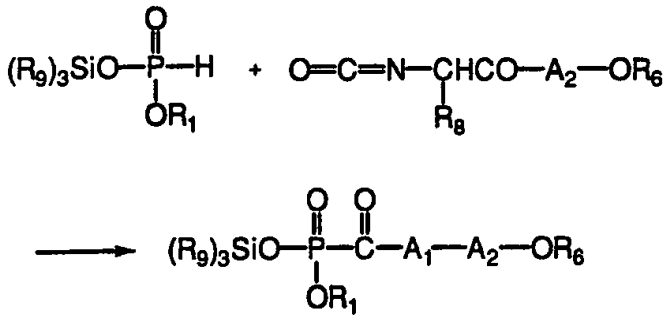


XVI

with a compound X-Si$(R_9)_3$, wherein A_1, A_2, R_1, R_6, R_9, R_{10} and X have the meaning given above.

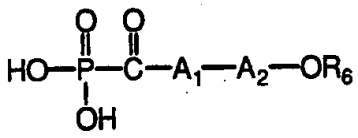
Preferably the silylation reagents are for example bromotrimethylsilane at -20°C to 50°C for 0.5 to 20 hours, or alternatively for example chlorotrimethylsilane at 20°C to reflux temperature for several days. The phosphonic acid diesters of formula XVI may be prepared by methods analogous to those described in A-J.

Alternatively, the silyl esterified phosphonate group may be prepared by reacting a silyl phosphite with an isocyanate according to the formula



wherein R_1, R_6, R_8, R_9 A_1 and A_2 have the meaning given above. Preferentially, the reaction is carried out at 25°C to 150°C for 1 to 50 hours.

O. Monoesterification of a compound of the formula VII



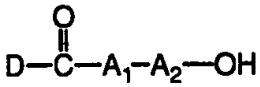
VII

with an alcohol R_1OH, wherein A_1, A_2, R_1 and R_6 have the meaning given above, followed by removal of the protecting group R_6, as described above, to give a compound of the formula II where R_2=H.

The esterification reaction is performed through the intermediary of activating agents known per se for the phosphorylation of alcohols. Examples of such methods are described for example by L. A. Slotin in Synthesis 1977, 737 and by H. Seliger and H. Kössel in Progress in the Chemistry of Organic Natural Products 32 (1971) 297.

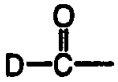
The phosphonic acids of formula VII are prepared by the methods described above in methods K and L.

Compounds of the general formula I



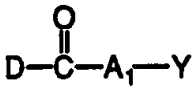
I

wherein



is a radical of a pharmaceutically active compound, D-COOH, and A_1 and A_2 have the meaning given above are prepared by known methods for the synthesis of peptide derivatives, as described for example in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XV/2, Die Herstellung der Peptidbindung, p. 1-453. Examples of such methods are the following.

P. Reacting a compound of the general formula XVIII



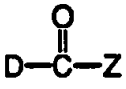
XVIII

wherein D, A_1, and Y have the meaning given above, with an amino acid derivative

$H\text{-}A_2\text{-}OR_6$

wherein A_2 and R_6 have the meaning given above, followed by removal of the protecting group R_6, as described above, to give a compound of the formula I.

The amino acid derivatives of formula XVIII are prepared by reacting a compound of the general formula XIX



XIX

wherein D has the meaning given above and Z has the meaning given Y, or may

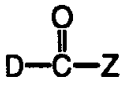
be any suitable leaving group such as for example Cl, Br, I or F, with an amino acid derivative

$$H\text{-}A_1\text{-}OR_6$$

wherein A_1, and R_6 have the meaning given above, followed by removal of the protecting group R_6, as described above, and introduction of the carboxyl-activating group Y as described for example in Houben-Weyl, Methoden der Organischen Chemie, Auflage 4, Band XV/2, Die Herstellung der Peptidbindung, p. 1-453.

If necessary, any reactive groups present in the moiety D of the pharmaceutically active compound D-COOH, such as for example hydroxyl groups, thiol groups and amino groups, may be protected by suitable protecting groups, as described for example in T.W. Greene & P.G.M Wuts, Protective Groups in Organic Synthesis, Second Edition, John Wiley, New York, 1991, prior to the reactions described above. Such protecting groups may then be removed, as described for example in the above reference, following completion of the reactions described above.

Q. Reacting a compound of the general formula XIX



XIX

wherein D and Z have the meanings given above, with a dipeptide derivative

$$H\text{-}A_1\text{-}A_2\text{-}OR_6$$

wherein A_1, A_2 and R_6 have the meanings given above, followed by removal of the protecting group R_6, as described above, to give a compound of the formula I.

If necessary, any reactive groups present in the moiety D of the pharmaceutically active compound D-COOH, such as for example hydroxyl groups, thiol groups and amino groups, may be protected by suitable protecting groups, as described for example in T.W. Greene & P.G.M. Wuts, Protective Groups in Organic Synthesis, Second Edition, John Wiley, New York, 1991, prior to the reactions described above. Such protecting groups may then be removed, as described for example in the above reference, following completion of the reactions described above.

Pharmaceutical compositions

The compounds of the present invention are suitably admixed with excipients to be formulated into capsules, tablets, suppositories and others such as suspensions and solutions. Using known pharmaceutical procedures oral formulations of capsules at doses of 50 mg to 1000 mg may be formulated.

For clinical use the compounds of the invention are formulated into pharmaceutical formulations for oral, parenteral and rectal administration. The pharmaceutical formulation contains the compound of the invention normally in combination with a pharmaceutically acceptable excipient. The excipient may be in the form of a solid, semi-solid or liquid diluent, or a capsule. These pharmaceutical preparations are a further object of the invention. Usually the amount of active compound is between 0.1-99% by weight of the preparation for oral as well as for other modes of administration.

In the preparation of pharmaceutical formulations containing the compounds of the present invention in the form of dosage units for oral administration the compound may be mixed with a solid, powdered carrier, such as lactose, saccharose, sorbitol, mannitol, starch, amylopectin, cellulose derivatives, gelatin, or another suitable carrier, stabilizing substances such as alkaline compounds e.g. carbonates, hydroxides and oxides of sodium, potassium, calcium, magnesium, and the like, as well as with lubricating agents such as magnesium stearate, calcium stearate,

sodium stearyl fumarate and polyethyleneglycol waxes. The mixture is then processed into granules or pressed into tablets. Granules and tablets may be coated with an enteric coating which protects the active compound from acid catalyzed degradation as long as the dosage form remains in the stomach. The enteric coating is chosen among pharmaceutically acceptable enteric-coating materials e.g. beeswax, shellac or anionic film-forming polymers and the like, if preferred in combination with a suitable plasticizer. To the coating various dyes may be added in order to distinguish among tablets or granules with different active compounds or with different amounts of the active compound present.

Soft gelatin capsules may be prepared with capsules containing a mixture of the active compound of the invention, vegetable oil, fat, or other suitable vehicle for soft gelatin capsules. Soft gelatin capsules may also be enteric-coated as described above.

Hard gelatin capsules may also contain the active compound in combination with a powdered carrier such as lactose, saccharose, sorbitol, mannitol, potato starch, amylopectin, cellulose derivatives or gelatin. The hard gelatin capsules may be enteric-coated as described above. Hard gelatin capsules may contain granules or enteric-coated granules of the active compound.

Dosage units for rectal administration may be prepared in the form of suppositories with the active substance mixed with a neutral fat base, or they may be prepared in the form of a gelatin rectal capsule which contains the active substance in a mixture with a vegetable oil, paraffin oil or other suitable vehicle for gelatin rectal capsules, or they may be prepared in the form of a dry micro enema, or they may be reconstituted in a suitable solvent just prior to administration.

Liquid preparations for oral administration may be prepared in the form of syrups or suspensions, e.g. solutions or suspensions containing from 0.2% to 20% by weight of the active ingredient and the remainder consisting of sugar or sugar

alcohols and a mixture of ethanol, water, glycerol, propylene glycol and/or polyethylene glycol. If desired, such liquid preparations may contain colouring agents, flavouring agents, saccharin and carboxymethyl or other thickening agents. Liquid preparations for oral administration may also be prepared in the form of a dry powder to be reconstituted with a suitable solvent prior to use.

In addition, using known pharmaceutical procedures, sustained release preparations at doses of 50 mg to 1000 mg, preferably 1000 mg may be formulated. Suitable sustained release formulations may include pharmaceutically acceptable excipients.

The typical daily dose of the active substance will depend on various factors such as for example the individual requirement of each patient, the route of administration and the disease. In general, oral dosages will be in the range of 50 to 20000 mg of active substance per day, preferably 200 mg to 15000 mg of active substance per day.

Experiments

Bioavailability studies

In vivo experiments were performed using male Wistar rats, breed U:WU rats. PFA or a derivative of PFA according to the present invention was administered orally or intravenously to the rat and whole blood samples (200 µl) were collected from the canulated jugular vein and stored in heparinised cups. Oral administration of DEPF-GlyPro and PFA was calculated relative to an intravenous dose of PFA in a cross-over design experiment in 4 animals. The obtained figures of results were treated statistically to determine the standard deviation. After centrifugation, 20.0 µl plasma samples were taken and diluted with 180 µl 1 mM pyrophosphoric acid and treated with about 20 mg activated charcoal. After intensive vortexing the samples were centrifuged for 15 min at 2000 g and the supernatant was brought into vials

for a sample processor (Waters WISP 710B).

Phosphonoformic acid plasma standards (ranging from 0.5 µM to 1 mM) prepared with rat plasma, were used to quantify the concentration of phosphonoformic acid in the samples.

Immediately after the workup procedure the samples were injected onto a HPLC system consisting of an LDC analytical ConstaMetric®3200 solvent delivery system with a built-in pulse dampener (Laboratory Data Control, Riviera Beach, Fl, USA), a WISP 710B autosampler, a Kratos Spectroflow 773 variable wavelength UV detector operating at 230 nm, and an ESA Coulochem®II electrochemical detector (ESA Inc, Bedford, MA, USA) with a model 5011 high sensitivity analytical cell. The potentials on the analytical cell were set at +0.75 and +0.95V for channels 1 and 2, resp. The mobile phase (flow rate 0.7 ml/min) was a pH 5.8 phosphate buffer-methanol (75:25, v:v) mixture with 1 mM tetrahexylammonium hydrogen sulfate as ion pair creator and 0.2 mM pyrophosphoric acid to prevent peak tailing. The total concentration of phosphate in the mobile phase was 43 mM. The analytical column, a Merck LiChrospher® 100 RP 18 (5µm; E Merck Nederland BV, Amsterdam, NL) was held at a constant temperature of 37°C. Under these conditions phosphonoformic acid showed a retention time of 8.5 min.

The oral absorption of the derivative of PFA was tested compared to the oral absorption of PFA in an aqueous solution. Both oral administrations were compared to an intravenous dose of PFA to calculate the absolute bioavailability (F_{abs}). All doses were equimolar (180 µmol/kg). Solutions for i.v. and p.o. administration were 120 mM in sterile normal saline with respect to PFA or DEPF-GlyPro. The results are given in Figure 1. From Fig. 1 it can be seen that the plasma concentration after oral administration of DEPF-GlyPro reaches a C_{max} of 0.18 mM as compared to C_{max} of 0.05 mM for PFA in aqueous solution. The bioavailability measured as F_{abs} is 0.89 for DEPF-GlyPro as compared to 0.19 for PFA in aqueous solution which corresponds to an over 4-fold increase. The time to

reach C_{max} was 1.15 h for DEPF-GlyPro and 1.25 h for PFA. For a compilation of results see Table 1.

Table 1

Compound	Dose (μmol/kg)	Route	AUC (mM•h)	F_{abs}
PFA	180	i.v.	0.57 ± 0.03	-
PFA	180	p.o.	0.11 ± 0.05	0.19 ± 0.04
DEPF-GlyPro	180	p.o.	0.51 ± 0.06	0.89 ± 0.08

In vitro intestinal transport study

Transport of DEPF-GlyPro across living intestinal tissue was performed in Ussing chambers (Ussing, H.H. and Zehran, K., Acta Physiol. Scand. 23:110-127(1951)) in the following way: Rat intestine (Wistar rats, breed U:WU) was stripped of its underlying muscle layer, placed between two Lucite® chambers and bathed on both sides with a TRIS-Ringer solution with a pH of 7.4 containing 10 mM glucose at the serosal side and 10 mM mannitol on the mucosal side. During transport studies tissue integrity was monitored using fluorescein as a fluorescent transport marker. The viability was monitored measuring potential difference and short-circuited current of the tissue.

After equilibration during 45 min PFA or DEPF-GlyPro was added to the donor side of the membrane at a concentration of 1 mM. For studies of the transport from the mucosal to the serosal side (m-to-s) the substance to be tested was added on the mucosal side and for transport in the opposite direction (s-to-m) it was added on the serosal side. At intervals of 30 min samples were taken from the acceptor phase up to 210 minutes. In order to maintain a constant volume, TRIS-Ringer solution with 10 mM glucose for the mucosal to serosal side studies and Tris-Ringer solution with 10 mM mannitol for the serosal to mucosal side studies was

added. The acceptor samples were assayed for PFA or DEPF-GlyPro using the HPLC assay described above.

Fig. 2 illustrates the transport of PFA and DEPF-GlyPro at a donor concentration of 1 mM during a period of 210 minutes over rat jejunum. A higher m-to-s (closed symbols) transport rate than s-to-m (open symbols) transport rate was observed. This suggests that in transport from the mucosal to serosal side another mechanism is involved in addition to passive diffusion. The acceptor samples were assayed for PFA or DEPF-GlyPro using the HPLC assay described above.

Example 1

Di-(O-ethyl)phosphonoformylglycylproline was prepared in four steps in the following way:

Step 1

Preparation of di-(O-ethyl)phosphonoformylglycine ethyl ester (DEPF-Gly-OEt; M_w 267).

Dioxane was refluxed overnight over cuprous chloride (CuCl) and freshly distilled before use. 6.3 Ml (47.9 mmol: 1.2 eq) trimethylsilyl azide (TMSA) was slowly added to 50 ml dioxane under an argon atmosphere. 5 Ml (39.7 mmol; 1 eq) ethyl malonyl chloride (EMC) was slowly added to this mixture under continuous stirring. After 15 minutes the temperature was raised to 90-100°C. Vigorous stirring of the yellow solution resulted in the escape of nitrogen bubbles, which stopped after 3 hours. Trimethylsilyl chloride (TMSC; b.p. 56-57°C) formed during the reaction distilled off. Then 6.2 ml (48.1 mmol; 1.2 eq) diethylphosphite (DEP) was added dropwise to the resulting reaction mixture and was refluxed for 1.5 hrs. Dioxane was removed under reduced pressure to give a thick yellow liquid (78% yield).

A sample was further purified by extraction from DCM to give a pale yellow liquid.

Bp 172°C;

n=1.398;

^{1}H-NMR (300 MHz, DMSO-d_6) δ(ppm) 1.1 (t, 6H), 1.3 (t, 3H), 4.45 (m, 6H), 3.6 (m, 2H); EI-MS; 267 (M/Z);

FAB-MS: 268 (M+1);

FT-IR (cm^{-1}): 3271 (m, NH), 3000 (s, CH_2/CH_3), 1752 (s, C=O aliphatic ester), 1662 (s, NHCO), 1520 (m, NHCO), 1250 (s, P=O), 1200 (s, P-O), 875 (s, CH_2/CH_3).

Step 2

Preparation of di-(O-ethyl)phosphonoformylglycine (DEPF-Gly; M_w 239).

0.782 g DEPF-GlyOEt was dissolved in 1 ml water. This mixture was poured into 2.0 ml 0.1M borate-buffer (pH 8) and 70 µl (200 units) porcine liver esterase (PLE; carboxylic ester hydrolase; EC 3.1.1.1) was added conforming with Sigma PLE product information. This mixture was stirred overnight keeping the pH at 8 by adding 0.1M NaOH-solution. The reaction was stopped by adding 5.0 ml dichloromethane (DCM) to the mixture which denaturated the esterase. After filtration of the brown precipitate the aqueous layer was extracted three times with DCM. The aqueous layer was neutralised and further purified by silica gel chromatography which yielded 62% of compound DEPF-Gly as a white solid.

UV:λ_{max}=208.3 nm;

Mp 160-162°C;

^{1}H-NMR (300 MHz, DMSO-d_6) δ(ppm) 1.2 (t, 6H), 4.1 (d, 4H), 3.85 (d, 2H), 8.7 (s, 1H);

FAB-MS: 240 (M+1);

FT-IR (cm^{-1}): 3520 (w, OH), 3271 (m, NH), 3000 (s, CH_2/CH_3, 1662 (s, NHCO), 1520 (m, NHCO), 1250 (s, P=O), 1200 (s, P-O), 875 (s, CH_2/CH_3).

Step 3

Preparation of di-(O-ethyl)phosphonoformylglycylproline methyl ester (DEPF-GlyPro-OMe; M_w 350).

A fine suspension of 400 mg DEPF-Gly was created in DCM by sonication and 250 mg (1.1 eq) 1-hydroxybenzotriazole (HOBT) was added to activate the carboxylic acid before coupling. The suspension was brought to 0°C in an ice-bath and a solution of 380 mg (1.1 eq) N, N'-dicyclohexylcarbodiimide (DCC) in DCM was added under vigorous stirring. After 1 hour of stirring a suspension of 277 mg (1 eq) L-proline methyl ester hydrochloride (Pro-OMe) and 193 mg (1 eq) N-ethylmorpholine (NEM) was added dropwise. The solution was stirred for 1 hour at 0°C and stirred overnight at room temperature. Dicyclohexylurea (DCU) which precipitated during the reaction was filtered off. The filtrate was extracted successively with a solution of saturated $NaHCO_3$, 2M citric acid, saturated $NaHCO_3$ solution and water. The organic layer was collected and DCM was removed under reduced pressure resulting in a red solid, yielding 52% DEPF-GlyPro-OMe.

M.p. 208-210°;

UV:λ_{max}=234 nm;

FAB-MS:351 (M+1);

^{1}H-NMR:(D_2O) δ (ppm): 1.3 (m,6H); 1.8 (m,2H); 2.4 (m,2H); 3.5 (t,2H); 3.7 (s,3H); 4,0 (d,2H); 4.2 (t,1H); 4.6 (m,4H).

Step 4

Preparation of di-(O-ethyl)phosphonoformylglycylproline (DEPF-GlyPro; M_w336).

10 Ml of a 1M NaOH solution in ethanol is added to 250 mg DEPF-GlyPro-OMe. The suspension is slowly stirred for 1 hour at room temperature and then neutralised by adding 10 ml 1M HCl with subsequent extraction with EtOAc. The

crude product was chromatographed on silica gel by elution with MeOH/H_2O (gradient 0-100%). 214 mg of DEPF-GlyPro was obtained (89%) as a white solid. The water content of the final product was 5.0%.

M.p. 215-216°C;

UV:λ_{max}=228 nm;

FAB-MS:337 (M+1);

^{1}H-NMR: (D_2O) δ (ppm): 1.3 (m,6H); 1.8 (m,2H); 2.4 (m,2H); 3.5 (t,2H); 4.0 (t,2H); 4.2 (t,1H); 4.6 (m,4H).

The following examples illustrate the preparation of pharmaceutical compositions of the invention. The active substance can be used as such or as a salt where such salts have suitable properties.

Example 2

Tablets

Each tablet contains:

active substance	300.0 mg
lactose	200.0 mg
maize starch	25.0 mg
gelatin	1.5 mg
talc	12.0 mg
magnesium stearate	1.5 mg

Example 3

Coated tablets

Tablets according example 2 are coated with an enteric coating solution with the following composition:

ethyl cellulose	120.0 g
propylene glycol	30.0 g
sorbitan monooleate	10.0 g
water	ad 1000.0 ml

The coating is carried out by a pouring procedure in a conventional coating pan or by spraying the tablets in a pan spray tablet coater.

Example 4

Gastric juice-resistant tablets

Tablets according example 2 are coated with a coating solution with the following composition:

cellulose acetate phthalate	120.0 g
propylene glycol	30.0 g
sorbitan monooleate	10.0 g
ethanol 95%	450.0 ml
acetone	q.s. ad 1000.0 ml

The coating is carried out by a pouring procedure in a conventional coating pan or by spraying the tablets in a pan spray tablet coater.

Example 5

Syrup

active substance	12.0 g
liquid glucose	30.0 g
sucrose	40.0 g
ascorbic acid	0.1 g
disodium edetate	10.0 mg
lemon essence	25.0 mg
purified water	ad 100.0 g

Example 6

Powder for suspension (Sachet)

Each sachet contains:

active substance	3.0 g
citric acid anhydrous	0.5 g
glucose	2.5 g

Example 7

Capsules

Each capsule contains:

active substance	300.0 mg
microcrystalline cellulose	150.0 mg
colloidal silicium oxide	5.0 mg

Example 8

Solution for injection

active substance	5.0 g
disodium edetate	2.5 mg
sodium chloride for isotonia	q.s.
hydrochloric acid to pH 6.5-7	
sterile water for injection	ad 100.0 ml

Example 9

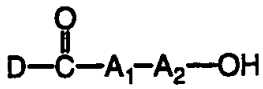
Suppositories

Each suppository contains:

active substance	500.0 mg
adeps solidus	q.s.

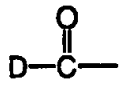
Claims

1. A compound of the formula I



I

wherein

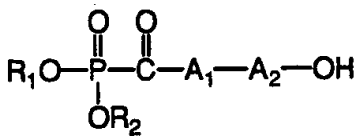


is a radical of a pharmaceutically active compound, D-COOH which is not an α-amino acid or peptide and which is able to form an amide bond with the N-terminal group of a dipeptide H-A_1-A_2-OH as defined below;

A_1 is an amino acid residue which is selected from glycyl, alanyl, valyl, norvalyl, leucyl, isoleucyl, norleucyl, phenylalanyl, tyrosyl, seryl, homoseryl, threonyl, cysteinyl, methionyl, tryptophyl, α-aspartyl, α-glutamyl, arginyl, lysyl, histidyl, ornithyl, prolyl or 4-hydroxyprolyl, either in the L- or in the D-configuration;

A_2 is an amino acid residue which is selected from prolyl, 4-hydroxyprolyl, phenylalanyl or tyrosyl, either in the L- or in the D-configuration; or physiologically acceptable salts thereof.

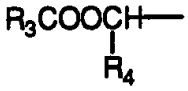
2. A compound of the formula II



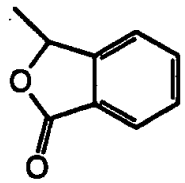
II

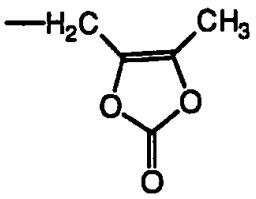
wherein A_1 and A_2 are as defined above in claim 1;

wherein R_1 and R_2 each independently are hydrogen; a straight or branched C_{1-6}-alkyl, C_{2-6}-alkenyl, C_{2-6}alkynyl, C_{3-8}-cycloalkyl, C_{3-8}-cycloalkyl-C_{1-6}-alkyl or C_{1-6}-alkoxy-C_{1-6}-alkyl group which is optionally substituted with hydroxy, amino, halogen or oxo; a benzyl group;

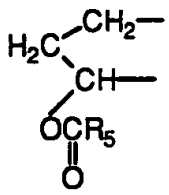
a group  or a group 

wherein R_3 is a straight or branched C_{1-6}-alkyl, C_{2-6}-alkenyl, C_{2-6}-alkynyl, C_{3-8}-cycloalkyl, C_{3-8}-cycloalkyl-C_{1-6}-alkyl or C_{1-6}-alkoxy-C_{1-6}-alkyl group which is optionally substituted with hydroxy, amino, halogen or oxo, and R_4 is hydrogen or a C_{1-4}-alkyl group;

a group  ;

a group  ;

or wherein R_1 and R_2 together form a group



wherein R_5 is a straight or branched C_{1-6}-alkyl or C_{1-6}-alkoxy group or physiologically acceptable salts thereof.

3. A compound according to claim 2 wherein R_1 and R_2 are ethyl.

4. A compound according to any of claims 1 to 3 wherein A_1 is a glycyl residue.

5. A compound according to any of claims 1 to 3 wherein A_1 is a L-alanyl residue.

6. A compound according to any of claims 1 to 5 wherein A_2 is a L-prolyl residue.

7. A compound according to any of claims 1 to 5 wherein A_2 is a L-4-hydroxyprolyl residue.

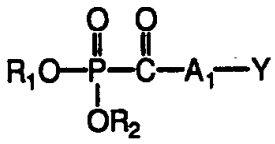
8. A compound according to any of claims 1 to 5 wherein A_2 is a L-phenylalanyl

residue.

9. A compound according to any of claims 1 to 5 wherein A_2 is a L-tyrosyl residue.

10. A process for the preparation of a compound of the formula II as defined in claim 2, characterized by

A. reaction of a compound of the formula III



III

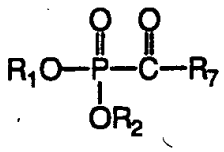
with an amino acid derivative

$H\text{-}A_2\text{-}OR_6$

wherein R_1, R_2, A_1 and A_2 have the meaning given above, R_6 is a suitable carboxyl protecting group, such as methyl, ethyl or benzyl, and Y is a hydroxyl group or a carboxyl-activating group such as azido, pentachlorophenoxy, 4-nitrophenoxy, succinimidoxy, benzotriazol-1-oxy, or imidazolyl, followed by removal of the protecting group R_6 to give a compound of the formula II, or

B. reaction of a compound of the formula IV

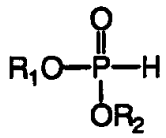
IV



IV

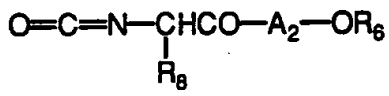
wherein R_1 and R_2 have the meaning given above and R_7 is an aliphatic, cycloaliphatic, araliphatic, aromatic or heterocyclic leaving group, such as C_{1-6}-alkoxy, C_{3-8}-cycloalkoxy, benzyloxy, phenoxy, 4-nitrophenoxy, imidazolyl or succinimidoxy, with a dipeptide derivative H-A_1-A_2-OR_6 wherein A_1, A_2 and R_6 have the meanings given above, followed by removal of the protecting group R_6 to give a compound of the formula II, or

C. reaction of a compound of the formula V



V

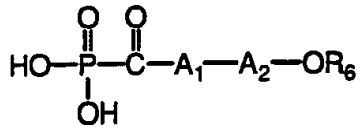
wherein R_1 and R_2 have the meaning given above, with a compound of the formula VI



VI

wherein A_2 and R_6 have the meaning given above and R_8 is the side chain specific for an amino acid residue A_1 as defined in claim 1, followed by removal of the protecting group R_6 to give a compound of the formula II, or

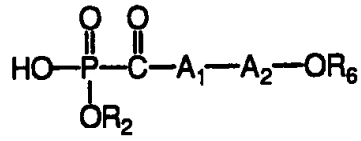
D. esterification of a compound of the formula VII



VII

with an alcohol R_1OH, wherein A_1, A_2, R_1 and R_6 have the meaning given above, followed by removal of the protecting group R_6 to give a compound of the formula II where R_1=R_2, or

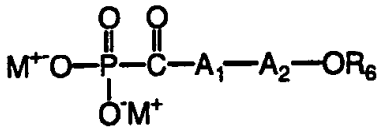
E. esterification of a compound of the formula VIII



VIII

with an alcohol R_1OH, wherein A_1, A_2, R_1, R_2 and R_6 have the meaning given above, followed by removal of the protecting group R_6 to give a compound of the formula II, or

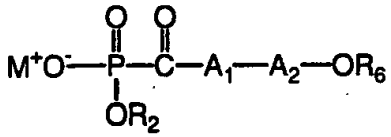
F. reaction of a compound of the formula IX



IX

with a compound R_1-X, wherein A_1, A_2, R_1 and R_6 have the meaning given above, M^+ is a cation such as Ag^+, Li^+, Na^+, K^+, Cs^+, Et_3NH^+ and $(i\text{-}Pr)_2NEtH^+$, and X is a halogen such as Cl, Br or I, followed by removal of the protecting group R_6 to give a compound of the formula II where R_1=R_2, or

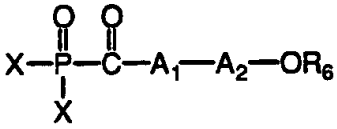
G. reaction of a compound of the formula X



X

with a compound R_1-X, wherein A_1, A_2, R_1, R_2, R_6, M^+ and X have the meaning given above, followed by removal of the protecting group R_6 to give a compound of the formula II, or

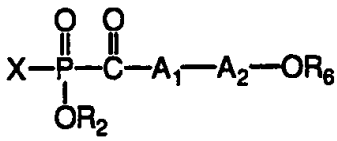
H. reacting a compound of the formula XI



XI

with an alcohol R_1OH, wherein A_1, A_2, R_1, R_6 and X have the meaning given above, followed by removal of the protecting group R_6 to give a compound of the formula II where R_1=R_2, or

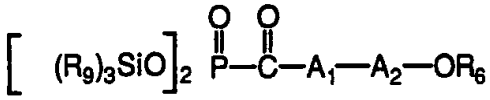
J. reacting a compound of the formula XII



XII

with an alcohol R_1OH, wherein A_1, A_2, R_1, R_2, R_6 and X have the meaning given above, followed by removal of the protecting group R_6 to give a compound of the formula II, or

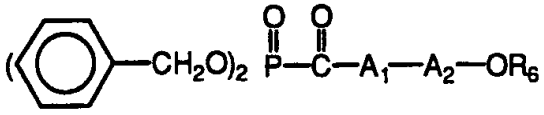
K. aqueous hydrolysis of a compound of the formula XIII containing two silylated phosphonate groups



XIII

wherein A_1, A_2 and R_6 have the meaning given above, and R_9 is an inert organic residue, for example methyl, followed by removal of the protecting group R_6 to give a compound of the formula II where R_1=R_2=H, or

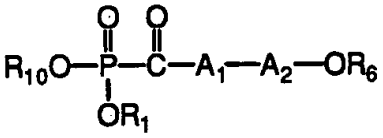
L. hydrogenation of a compound of the formula XV



XV

wherein A_1, A_2 and R_6 have the meaning given above, followed by removal of the protecting group R_6 to give a compound of the formula II where R_1=R_2=H, or

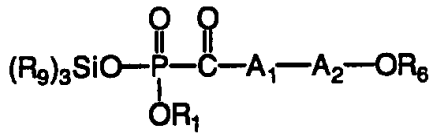
M. reacting a compound of the formula XVI



XVI

with iodide or bromide anion, wherein A_1, A_2, R_1 and R_6 have the meaning given above, and R_{10} has the meaning given R_1 and R_2, followed by removal of the protecting group R_6 to give a compound of the formula II where R_2=H, or

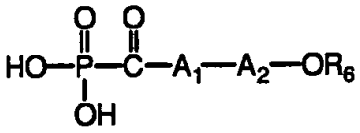
N. aqueous hydrolysis of a compound of the formula XVII containing one silylated phosphonate group



XVII

wherein A_1, A_2, R_1, R_6 and R_9 have the meaning given above, followed by removal of the protecting group R_6 to give a compound of the formula II where R_2=H, or

O. monoesterification of a compound of the formula VII



VII

with an alcohol R_1OH, wherein A_1, A_2, R_1 and R_6 have the meaning given above, followed by removal of the protecting group R_6 to give a compound of the formula II where R_2=H.

11. A compound according to any of claims 1 to 9 for use in therapy.

12. A compound according to any of claims 1 to 9 wherein D-COOH is an antiviral drug for use in the treatment of viral infections.

13. A compound according to any of claims 1 to 9 wherein D-COOH is an antiviral drug for use in the treatment of herpesvirus infections.

14. A compound according to any of claims 1 to 9 wherein D-COOH is an

antiviral drug for use in the treatment of HIV infections including the state of AIDS.

15. A compound according to claim 1, wherein D-COOH is an antibacterial drug for use in the treatment of bacterial infections.

16. A compound according to claim 1 wherein D-COOH is an analgesic drug for use in the treatment of pain.

17. A compound according to claim 1 wherein D-COOH is an antirheumatic drug for use in the treatment of arthritis.

18. A compound according to claim 1, wherein D-COOH is an antiphlogistic drug for use in the treatment of inflammatory diseases.

19. A compound according to claim 1, wherein D-COOH is an oncolytic drug for use in the treatment of tumors.

20. A compound according to claim 1, wherein D-COOH is a drug of the prostaglandin group for use in the control of acid secretion in the stomach.

21. A compound according to claim 1, wherein D-COOH is a drug of the prostaglandin group for use in the control of smooth muscle contractions in the uterus.

22. A compound according to claim 1 wherein D-COOH is a drug of the prostaglandin group for use in the treatment of asthma.

23. A compound according to claim 1, wherein D-COOH is a diuretic drug for use in increasing diuresis.

24. The use of a compound according to any of claims 1 to 9 wherein D-COOH is an antiviral drug in the manufacture of a formulation for the treatment of viral infections.

25. The use of a compound according to any of claims 1 to 9 wherein D-COOH is an antiviral drug in the manufacture of a formulation for the treatment of herpesvirus infections.

26. The use of a compound according to any of claims 1 to 9 wherein D-COOH is an antiviral drug in the manufacture of a formulation for the treatment of HIV including the state of AIDS.

27. The use of a compound according claim 1, wherein D-COOH is an antibacterial drug in the manufacture of a formulation for the treatment of bacterial infections.

28. The use of a compound according to claim 1, wherein D-COOH is an analgesic drug in the manufacture of a formulation for the treatment of pain.

29. The use of a compound according to claim 1, wherein D-COOH is an antirheumatic drug in the manufacture of a formulation for the treatment of arthritis.

30. The use of a compound according to claim 1, wherein D-COOH is an antiphlogistic drug in the manufacture of a formulation for the treatment of inflammatory diseases.

31. The use of a compound according claim 1, wherein D-COOH is an oncolytic drug in the manufacture of a formulation for the treatment of tumors.

32. The use of a compound according to claim 1, wherein D-COOH is a drug of the prostaglandin group in the manufacture of a formulation for the control of acid

secretion in the stomach.

33. The use of a compound according to claim 1, wherein D-COOH is a drug of the prostaglandin group in the manufacture of a formulation for the control of smooth muscle contraction in the uterus.

34. The use of a compound according to claim 1 wherein D-COOH is a drug of the prostaglandin group for use in the manufacture of a formulation for the treatment of asthma.

35. The use of a compound according to claim 1, wherein D-COOH is a diuretic drug in the manufacture of a formulation for the increasing diuresis.

36. A method for the treatment of herpesvirus infection wherein a therapeutically active amount of a compound according to any of claims 1 to 9 wherein D-COOH is an antiviral drug is administered to a mammal in the need of such treatment.

37. A method for the treatment of HIV infections including AIDS wherein a therapeutically active amount of a compound according to any of claims 1 to 9 wherein D-COOH is an antiviral drug is administered to a mammal in the need of such treatment.

38. A method for the treatment of bacterial infections wherein a therapeutically active amount of a compound according to claim 1, wherein D-COOH is an antibacterial drug is administered to a mammal in the need of such.

39. A method for the treatment of pain wherein a therapeutically active amount of a compound according to claim 1, wherein D-COOH is an analgesic drug is administered to a mammal in the need of such treatment.

40. A method for the treatment of arthritis wherein a therapeutically active amount

of a compound according to claim 1, wherein D-COOH is an antirheumatic drug is administered to a mammal in the need of such treatment.

41. A method for the treatment of inflammatory diseases wherein a therapeutically active amount of a compound according to claim 1, wherein D-COOH is an antiphlogistic drug is administered to a mammal in the need of such treatment.

42. A method for the treatment of tumors wherein a therapeutically active amount of a compound according to claim 1, wherein D-COOH is an oncolytic drug is administered to a mammal in the need of such treatment.

43. A method for the control of acid secretion in the stomach wherein a therapeutically active amount of a compound according to claim 1, wherein D-COOH is a drug of the prostaglandin group is administered to a mammal in the need of such treatment.

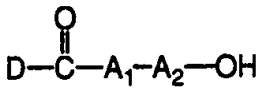
44. A method for the control of contractions in the uterus wherein a therapeutically active amount of a compound according to claim 1, wherein D-COOH is a drug of the prostaglandin group is administered to a mammal in the need of such treatment.

45. A method for the treatment of asthma wherein a therapeutically active amount of a compound according to claim 1 wherein D-COOH is a drug of the prostaglandin group is administered to a mammal in the need of such treatment.

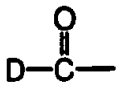
46. A method for increasing diuresis wherein a therapeutically active amount of a compound according to claim 1, wherein D-COOH is a diuretic drug is administered to a mammal in the need of such treatment.

47. The use of a dipeptide moiety $-A_1-A_2-OH$ in the design of prodrugs of the general formula I

I

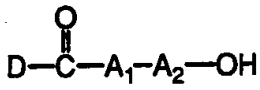


wherein A_1 is defined as in claim 1, and
wherein A_2 is defined as in claim 1, and
wherein



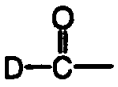
is defined as in claim 1, for increasing the uptake via the gastrointestinal tract into the blood of the parent drug D-COOH.

48. The use of a dipeptide residue -A_1-A_2-OH as a moiety to be linked by an amide bond in a compound of the general formula I



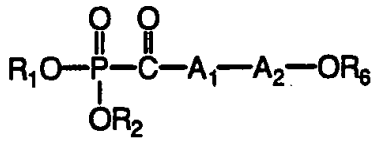
I

wherein A_1 and A_2 are defined as in claim 1, and
wherein



is defined as in claim 1 for increasing the uptake via the gastrointestinal tract into the blood of the parent drug D-COOH.

49. A compound of the formula



wherein R_1, R_2, R_6, A_1 and A_2 have the meaning given in claims 1, 2 and 10.

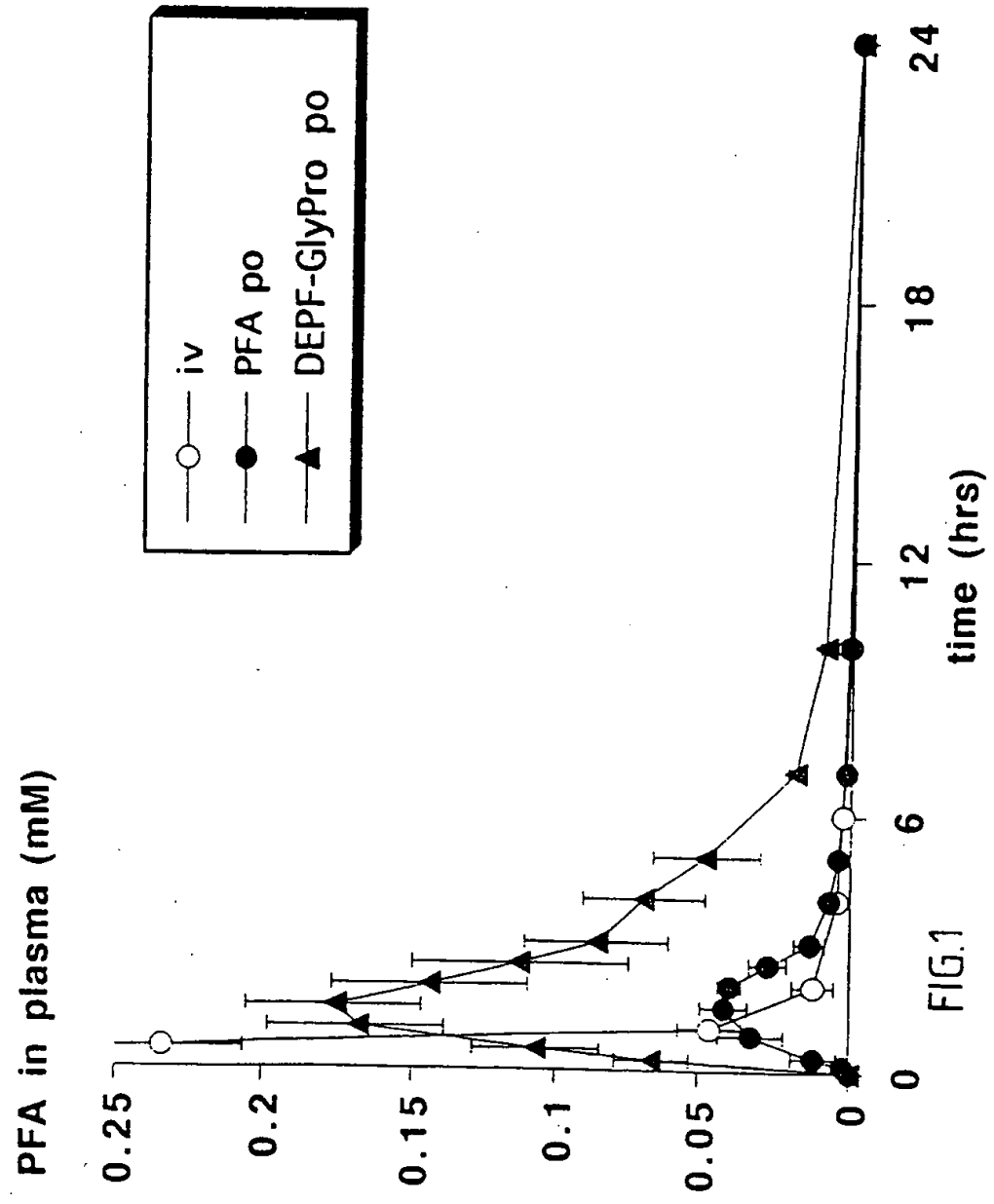

iv
PFA po
DEPF-GlyPro po
PFA in plasma (mM)
0.25
0.2
0.15
0.1
0.05
0
0
6
12
18
24
time (hrs)

FIG.1

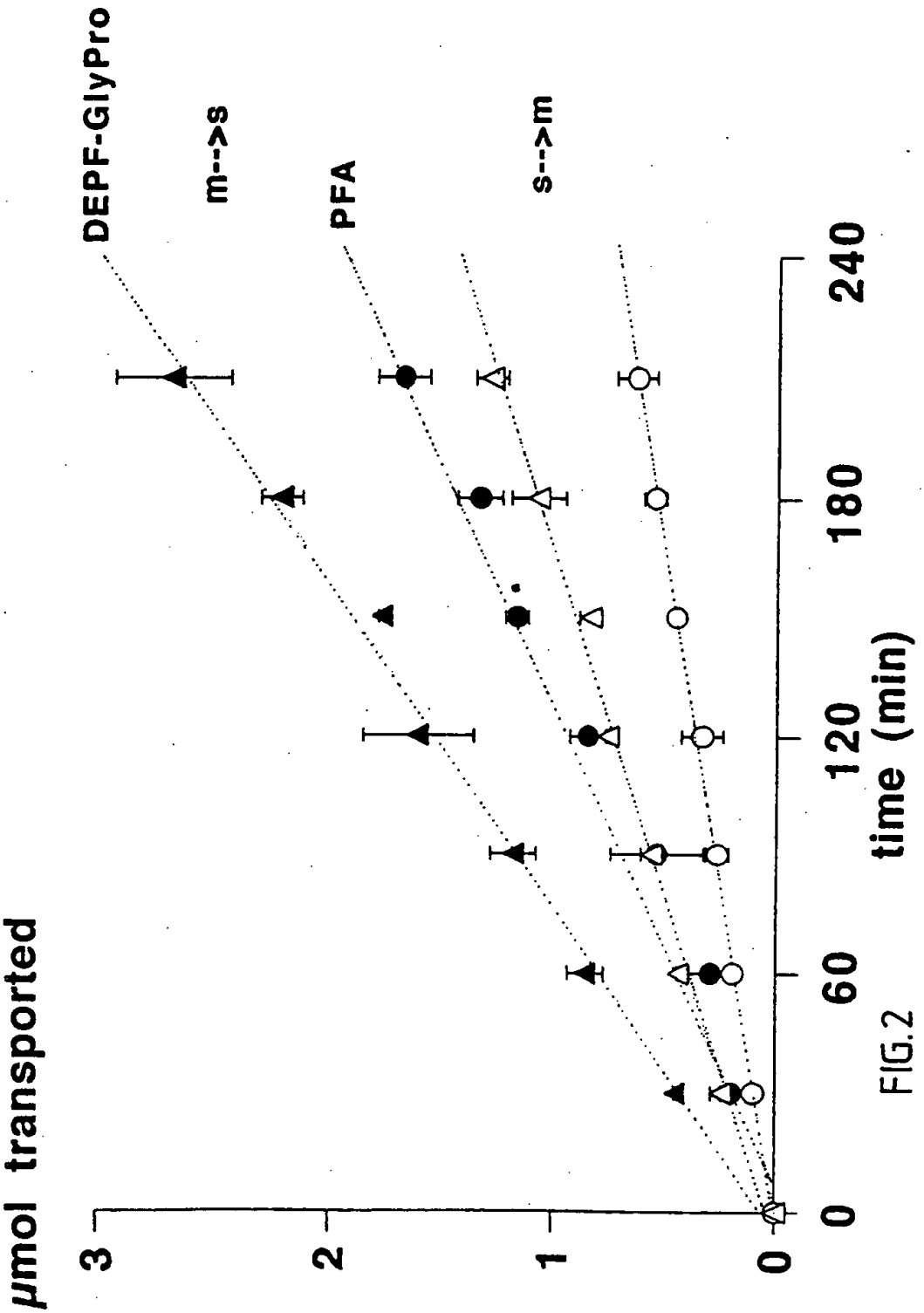

DEPF-GlyPro
m-->s
PFA
s-->m
µmol transported
3
2
1
0
0
60
120
180
240
time (min)

FIG.2

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 93/00997

A. CLASSIFICATION OF SUBJECT MATTER

IPC6: C07K 5/06, A61K 47/48
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC6: A61K, C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

SE,DK,FI,NO classes as above

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

CA, WPIDS, REG.FILE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO, A1, 8300146 (FLORK, MICHEL), 20 January 1983 (20.01.83), page 3, line 22 - page 4, line 10 ---	1-35,47,48
X	Patent Abstracts of Japan, Vol 10,No 339, C-385, abstract of JP, A, 61-145198 (FUJI YAKUHIN KOGYO K.K.), 2 July 1986 (02.07.86) ---	1-11
A	EP, A1, 0276436 (F. HOFFMANN-LA ROCHE & CO), 3 August 1988 (03.08.88) ---	1-35,47-49
A	DE, C2, 2559928 (DAIICHI SEIYAKU CO. LTD.), 16 August 1984 (16.08.84) ---	1-35,47-49

[X] Further documents are listed in the continuation of Box C. [X] See patent family annex.

* Special categories of cited documents:
"A" document defining the general state of the art which is not considered to be of particular relevance
"E" erlier document but published on or after the international filing date
"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
"O" document referring to an oral disclosure, use, exhibition or other means
"P" document published prior to the international filing date but later than the priority date claimed
"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"X" document of particular relevance: the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"Y" document of particular relevance: the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
5 July 1994	11 -07- 1994
Name and mailing address of the ISA/ Swedish Patent Office Box 5055, S-102 42 STOCKHOLM Facsimile No. +46 8 666 02 86	Authorized officer Elisabeth Carlborg Telephone No. +46 8 782 25 00

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 93/00997

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	DE, A1, 4022267 (DEGUSSA AG), 16 January 1992 (16.01.92) --	1-35,47-49
A	EP, A1, 0407017 (TAISHO PHARMACEUTICAL CO. LTD.), 9 January 1991 (09.01.91) --	1-35,47-49
A	WO, A1, 9215608 (LACER S.A.), 17 Sept 1992 (17.09.92) --	1-35,47-49

INTERNATIONAL SEARCH REPORT

International application No.
PCT/SE 93/00997

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.: 36-46
because they relate to subject matter not required to be searched by this Authority, namely:
See PCT Rule 39.1 (iv): Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT
Information on patent family members

28/05/94

International application No.
PCT/SE 93/00997

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO-A1- 8300146	20/01/83	CA-A- 1190243	09/07/85
		CH-A,B- 665645	31/05/88
		EP-A,B- 0069674	12/01/83
		SE-T3- 0069674	
EP-A1- 0276436	03/08/88	SE-T3- 0276436	
		AU-B- 606901	21/02/91
		AU-A- 8217687	16/06/88
		DE-A,T- 3786250	22/07/93
		IL-A- 84766	27/02/94
		JP-A- 63156796	29/06/88
		US-A- 4885283	05/12/89
		US-A- 5006651	09/04/91
		ZA-A- 8709230	15/06/88
DE-C2- 2559928	16/08/84	CA-A- 1047029	23/01/79
		DE-A,B,C 2557145	24/06/76
		FR-A,B- 2294694	16/07/76
		JP-A- 51115438	12/10/76
		JP-B- 58010379	25/02/83
		NL-A- 7514765	22/06/76
		SE-B,C- 410596	22/10/79
		SE-A- 7514308	21/06/76
		US-A- 4025644	24/05/77
		US-A- 4057629	08/11/77
		US-A- 4097608	27/06/78
		JP-C- 1182324	09/12/83
		JP-A- 51115440	12/10/76
		JP-B- 58011425	02/03/83
		JP-A- 51125240	01/11/76
		JP-A- 51125241	01/11/76
DE-A1- 4022267	16/01/92	EP-A- 0466030	15/01/92
EP-A1- 0407017	09/01/91	CA-A- 2016261	18/11/90
		JP-A- 3072478	27/03/91
		US-A- 5068354	26/11/91
WO-A1- 9215608	17/09/92	AU-A- 1278392	06/10/92
		EP-A- 0500989	02/09/92